Seasonal abundance of the stable fly *Stomoxys calcitrans* in south west England

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Abstract. The stable fly, *Stomoxys calcitrans* (L.), is a cosmopolitan biting fly of both economic and welfare concern, primarily due to its painful bite which can cause blood loss, discomfort and loss of productivity in livestock. Between June and November in 2016 and May and December in 2017, Alsynite sticky-traps were deployed at four Donkey Sanctuary sites in south west England, which experience recurrent seasonal biting fly problems. The aim was to evaluate the seasonal dynamics of the stable fly populations and the risk factors associated with abundance. In total, 19,835 *S. calcitrans* were trapped during the study period. In both years abundance increased gradually over summer months to peak in late August/ September. There were no relationships between seasonally detrended abundance and any climatic factors. Fly abundance was significantly different between sites and population size was consistent between years at three of the four sites. The median chronological age, determined by pteridine analysis of flies caught live whilst blood feeding, was 4.67 days (IQR 3.8-6.2 days) in males and 6.79 days (IQR 4.8-10.4 days) in females; there was no significant, consistent change in age or age structure over time suggesting that adult flies emerge continuously over the summer, rather than in discrete age-related cohorts. The data suggest that flies are more abundant in the vicinity of active animal facilities, but the strong behavioural association between flies and their hosts means that they are less likely to be caught on traps where host availability is high. The implications of these results for fly management are discussed.

Key words: Biting flies; Climate; Hosts; Pest Management; Alsynite Traps; Donkey

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Introduction

The stable fly, *Stomoxys calcitrans* (Diptera: Muscidae) is a synanthropic muscid of economic importance. Both sexes are obligatory blood-feeders and individuals may feed more than once a day, commonly feeding on the lower parts of the host's body and often probing repeatedly (Cortinas & Jones, 2006). Its painful bite leads to blood-loss, irritation and reduced grazing and it is therefore a species of welfare concern, particularly for cattle and equids (Cortinas & Jones, 2006; Salem *et al.,* 2012). Stable flies are not generally thought to be important biological vectors of human or animal pathogens, although in some areas they may act as mechanical vectors of pathogens, such as *Trypanosoma evansi* and *Dermatophilus congolensis* (Hall & Wall, 2004).

Stomoxys calcitrans breed in a wide range of media rich in organic material, including decomposing vegetation and straw, hay feeding sites and grass or leaves contaminated with urine or manure (Broce *et al.,* 2005; Cook *et al.,* 2018). Therefore breeding sites on livestock farms are usually abundant, and this, combined with the ready availability of hosts, can result in problematic populations (Hafez & Gamal-Eddin, 1959). The economic threshold for *S. calcitrans* is generally considered to be as low as 10 flies/animal and stable flies have been estimated to cost the US cattle industries over $2 billion a year due to reduced annual milk production and reduced weight gain (Taylor *et al.*, 2012).

Trapping approaches to stable fly sampling are well established and the potential use of trapping systems for fly population management is an issue of growing importance, particularly with regard to the need to reduce dependence on neurotoxic insecticides. Adult *S. calcitrans* show pronounced behavioural responses to reflectance of shorter wavelengths and a phototactic response to UV (Agee & Patterson, 1983). In the field adults assemble and insolate on sunlit light-coloured objects (Gibson & Torr, 1999); they largely remain in areas of strong sunlight and mainly bite out of doors, although they will follow animals inside and attempt to feed (Warnes & Finlayson, 1987). The response to specific wavelengths can be manipulated in the design of traps, and the incorporation of UV reflective fiberglass panels (e.g. Alsynite®) has proved particularly effective (Beresford & Sutcliffe, 2006). Traps made from Alsynite, a translucent fiberglass, were originally developed by Williams (1973) and have subsequently become widely used (Gersabeck & Merritt, 1983; Broce *et al.*, 1991). Broce *et al.* studied the landing pattern of stable flies on Alsynite fly traps, and found that wind direction and height of the trap affected where flies landed; a higher wind speed led to increased fly capture on the lower areas of the trap (1991). The Alsynite panels are usually covered by a sticky sleeve although this may reduce the catch (Hogsette & Ruff, 1990); nevertheless sticky sleeves are likely to remain the principal sticky substrate used for fly trapping for practical reasons.

The aims of the present study were to use sticky Alsynite traps to quantify the phenology of stable fly populations feeding on large populations of donkeys on sites in south west England managed by an animal welfare charity, and to consider the management factors that contribute to fly abundance with regard to improved management of stable flies. Observations have suggested that the level of attack and biting activity by *S. calcitrans* differs between individual hosts and is dependent on the reactions of the host when under attack. For example, cattle hosts which respond vigorously by tail flicks, foot stamps and head-swings have been shown to suffer less from attack by *S. calcitrans* than more placid individuals (Warnes & Finlayson, 1987). The outward response of donkeys to pain is considered to be subtle in comparison to other species, and the minimal response they display in relation to discomfort such as that caused by stable fly bites may mean that they are readily bitten, and blood-feeding may be a major welfare issue for these animals (Burden & Thiemann, 2015).

Methods

Study populations

The study took place between June 2016 and December 2017 at four rural sites, managed by an animal rescue charity (The Donkey Sanctuary) based near Sidmouth, in south west England. Sites ranged between altitudes of 83 m and 241 m above sea level, and were composed largely of fenced pasture and some mixed woodland. The size of the four sites was 55.85 ha, 60.99 ha, 72.22 ha and 80.35 ha, and the mean donkey populations over the two years of the study were 309, 251, 370 and 576 for sites 1 to 4, respectively. During the period of the study, at all sites, groups of donkeys were able to move freely between outdoor enclosures and indoor barns. The barns were generally similar in structure, layout and animal management regimen. Barns with a straw floor covering were completely cleared out approximately every two weeks; during this interval no manure was removed but fresh straw was added. At site 1, manure and bedding were collected at a single central permanent dung heap within a concrete-lined enclosure, while at sites 2, 3 and 4 soiled bedding and manure were stored temporarily on trailers at a range of locations at each site, from where it was removed intermittently. Persistent nuisance summer populations of *S. calcitrans* had been reported previously at all sites.

Fly collection

Cylindrical Alsynite fiberglass traps (Olson Products Inc., Medina, USA) were used for population sampling. The traps measured 30 cm in height and approximately 20 cm in diameter, and were covered with a replaceable sticky sleeve (Olson Products Inc., Medina, USA). The traps were fixed to wooden stakes which were driven into the ground, so that the bottom of each cylinder was approximately 45–60 cm above the ground.

In 2016, trapping was carried out between June and November. A total of 40 Alsynite traps were placed across the four sites. The traps were placed near barns, in open fields, against hedgerows or near manure heaps. The sticky sleeves were left on each trap for one week at a time before being removed and taken to the laboratory for examination. The following week new sticky sleeves were attached to the traps; in 2016 the traps were active for one week every fortnight. In 2017, trapping was carried out between May and December using only two traps at each of the four sites. Sticky sleeves were removed and replaced every week, so that flies were trapped continuously.

Once removed, sticky sheets were taken to the laboratory and captured *S. calcitrans* were identified. Daily weather data (max and min temperature, humidity and wind speed) for the Exeter area for both years were obtained online (www.wunderground.com). Trap coordinates and height above sea level were recorded using a hand-held GPS tracker (GPSmap 76CSx, Garmin Ltd., Schaffhausen, Switzerland). The distances between traps and occupied barns were measured using Google Maps (www.google.com/maps), and the number of donkeys in each barn was recorded by Donkey Sanctuary staff at the sites.

Pteridine analysis

To assess the age of flies caught in the field during 2017, the fluorescence of accumulated pteridine pigments was measured. The procedure for measuring levels of pteridine in *S. calcitrans* was adapted from the method of Mail *et al*. (1983). First a standard curve of pteridine levels in the heads of known-age laboratory-reared *S. calcitrans* was produced. For this a laboratory colony of *S. calcitrans*, derived from the Pirbright Institute, Surrey, was maintained at 25°C, 60% R.H., and under an 18:6 h light: dark regime. Adults were fed on citrated pig blood and larvae were fed on crushed and moistened dog biscuits and brewer's yeast powder.

Flies of known sex and age were collected from the colony, their heads were removed and homogenised using a glass rod in 500 μl of 0.05 m Tris-HCL buffer (pH 8.0). The homogenate was then centrifuged for 5 min at 5000 rpm, before the resulting supernatant was immediately measured for fluorescence using a FLUOstar OPTIMA microplate reader (BMG LABTECH, Aylesbury, UK) with an emission wavelength of 450 nm and an excitation wavelength of 340 nm. For each fly analysed, the posterior cross-vein (dm-cu) between the 4th and 5th longitudinal wing veins of the left wing was measured under a binocular microscope as an index of size. Pteridine concentrations were then expressed as Relative Fluorescence Unit (RFU) values divided by the cross-vein length, to correct for differences in fly size. To assess the effects of temperature on pteridine accumulation, the analysis was repeated with groups of 8-day-old *S. calcitrans* maintained in the laboratory at 20, 25 and 30 °C.

In 2017, live *S. calcitrans* were collected for pteridine analysis on seven separate occasions between August and October at approximately weekly intervals. Clear plastic tubes (69 mm by 28 mm in diameter) were used to trap *S. calcitrans* on the legs of donkeys at site 3. The tubes were quickly placed over flies which had settled on the leg of a donkey and begun to feed and a lid was immediately fastened onto the tube containing the fly once removed from the donkey's leg. Each trapping session lasted for 20-30 min. The captured *S. calcitrans* were returned to the laboratory within 24 h of capture and were subsequently frozen at -20 °C and maintained in the dark until required for pteridine assay.

Statistical analysis

Count data, expressed as catch per trap per week (+1) were $\log_{10}$ transformed to normalize the variance and subjected to statistical analysis using correlation, polynomial regression, multiple regression or analysis of variance and Tukey multiple range tests as detailed below (Statgraphics, version 16.1.03, StatPoint Technologies Inc., Warrenton, USA). Comparison of male and female fly ages were undertaken using a Mann-Whitney U-test, differences in age structure were compared using a Kolmogorov-Smirnov test and changes in population age-structure over time used a Kruskal-Wallis test (Statgraphics, version 16.1.03, StatPoint Technologies Inc., Warrenton, USA).

Results

Fly abundance

In total, 19,835 *S. calcitrans* were trapped during the two year study period. In both years the data showed a consistent gradual increase over summer to peak in late August/ September (weeks 22-28) (Fig. 1). To allow for seasonal changes, a third order polynomial was fitted to the $\log_{10}$ catch (+1) per trap per week data and then abundance was detrended by subtracting the regression line from the data. No significant relationships between temperature, humidity and wind speed and the detrended data could be detected by multiple regression analysis. Fly abundance was significantly different between the sites (ANOVA, $F_{3,732}$=28, P=0.001), with Tukey multiple range tests showing that site 3 consistently had the highest abundance whereas there was no significant difference between sites 1 and 4. Fly abundance shows no relationship with mean donkey numbers at each site. There was a significant interaction between site and year ($F_{3,1,732}$=4.75, P=0.003); however, this was the result of the difference in the numbers caught in the two years at site 1. In contrast, the abundance of *S. calcitrans* was relatively consistent on the other three sites between years (Fig. 2). Correlation of the mean log_{10} catch (+1) per trap per week at each site with that of every other, to determine whether the weekly changes in abundance followed the same pattern, shows that the weekly changes in abundance were significantly correlated in four cases (r=0.36, P=0.05; 0.37, P=0.05; 0.41, P=0.05; 0.68, P=0.001; for sites 1x2, 1x3, 1x4, and 2x4 respectively, n=30), but not for sites 2x3 or 3x4 where there were no significant correlations (r=0.24, P=0.2; r=0.24, P=0.2, respectively). To identify risk factors associated with fly abundance, the distance of each trap to a barn occupied by donkeys, size of each occupied barn, the average numbers of donkeys within 50 and 100 m of each trap and the presence or absence of a permanent dung heap on site were entered as independent variables in a multiple regression analysis with log_{10} catch per trap per week (+1) as the dependent variable. Only data from weeks 10 to 30 was used in this analysis to ensure comparability between years. Only the distance to the nearest occupied barn, the average number of donkeys within a radius of 50 m and the presence or absence of a managed dung heap on site were significantly associated with catch ($F_{2,708}$=13.95, P=0.001) but the variance explained by the model was low (r^2=6.64%). The catch of *S. calcitrans* was significantly higher at traps that were closer to barns occupied by donkeys (t_{708} = 3.06, P=0.02), trap catch declined with increasing numbers of donkeys within a 50 m radius (t_{708} = 4.16, P=0.001) and was greater on sites with multiple temporary dung storage sites than the site with a single permanent managed dung heap (t_{708} = 3.17, P=0.002).

Pteridine analysis

For laboratory-derived flies, RFU corrected for size showed highly significant multiplicative relationships with chronological age (Fig. 3), for both males and females. The accumulation of pteridine was temperature dependent, so a correction for the ambient temperature recorded in the field over the week prior to the capture of each fly was undertaken when calculating the age of field-derived flies (as detailed by Wall *et al.*, 1991). For field-derived flies collected whilst blood feeding, the median age of males was 4.67 days (Interquartile range (IQR) 3.8-6.2 days) and the median age of females was 6.79 days (IQR 4.8-10.4 days) (Fig. 4) , this difference was significant (Mann-Whitney U-test, n=207, W=7513, P=0.001) and the age structure of male and female populations was also significantly different with a greater tail of older female flies (Kolmogorov-Smirnov DN=2.38, n=207, P=0.001). However, there was no significant, consistent change in age or age structure over time (Kruskal-Wallis, H=8.61, n=207, P=0.19).

Discussion

The abundance of *S. calcitrans* has been shown to have a strongly seasonal pattern in many parts of its range. In Florida, USA, stable fly populations were recorded as abundant from November to June, peaking in April (Machtinger *et al.*, 2016). In Alberta, Canada, stable fly numbers have been shown to peak in August and September, though the flies are active from May through to October (Lysyk, 1993). In New Zealand *S. calcitrans* were found to be most active from January to May (Heath, 2002). Strong relationships between stable fly population dynamics and climatic variables such as temperature and precipitation have been identified in several studies (Taylor *et al.,* 2007; Skovgård & Nachman, 2012; Lysyk, 1993). In a 13 year study, Taylor *et al.* found that temperature and precipitation were able to explain 72% of the seasonal trap catch variance (2017). However, it is often difficult to distinguish underlying seasonal patterns of changing abundance from confounding seasonal changes in weather, hence it has been argued that detrending abundance data before relating it to climatic factors is essential (Cruikshank & Wall, 2002). In contrast to other studies, when detrended abundance data were used here, no relationship with temperature, humidity or wind speed was detected. However, a strong seasonal pattern was observed, and this pattern was repeated in the two years of the study and at all sites, with the stable fly population increasing gradually from early summer to autumn, peaking in late August/September. One potential contribution to the lack of relationship between stable fly abundance and climatic variables in the current study, may be that at the sites studied, a large proportion of the fly population emerged from indoor (barn) breeding sites where they would be less affected by temperature and precipitation than flies breeding outdoors.

The pteridine analysis showed that the adults could survive for 3 to 4 weeks, but the majority were less than 1 week old. No significant change in age structure was observed over time, suggesting that rather than discrete generations of flies, there was continuous emergence of adults throughout the summer as the population increased. Hence, because individual female *S. calcitrans* oviposit batches of between 20 to 100 eggs (Showler & Osbrink, 2015), and are likely to oviposit only once or twice in a lifetime in the field, the rate of population growth is relatively slow, as seen here.

The fact that the difference in abundance between sites was relatively consistent between both years of the study suggests that stable fly numbers may be related to consistent differences between sites in factors such as microclimate, habitat or animal management practices. Previous work demonstrated that when a trap was placed between host cattle and a group of trees, it caught significantly more stable flies than a trap which was placed near the cattle but out in the open, suggesting there was movement of flies between the hosts and the shelter trees (Guo *et al.*, 1998). It is possible that habitat differences that were not measured here, such as tree cover, water availability or surrounding agricultural land use, may have influenced the observed variation in stable fly abundance between sites

To assess potential factors that might facilitate higher fly abundance, a wide variety of factors were measured, but only the distance between the trap and the nearest occupied barn, the mean number of donkeys within a 50 m radius and the manure management strategy were significantly associated with trap catch. The distance between the trap and the nearest occupied barn were negatively correlated, with traps nearer occupied barns having a higher catch, as would be expected in flies which are strongly associated with animal housing facilities. This abundance-distance relationship has been observed previously for stable flies; abundance was shown to decrease geometrically with increasing distance from host cattle pens (Guo *et al.*, 1998). However, the relationship between trap catch and the number of animals within a 50 m radius was also negatively related: higher donkey numbers being associated with lower catches. This apparently contradictory result may be due to the fact that where large numbers of hosts are present, flies are likely to be strongly localised around the animals and less likely to be attracted to traps. Close association with host animals has been shown to be directly associated with fecundity in stable flies, with females provided with blood *ad libitum* producing more than 55% more eggs than females offered blood just once a day (Jones *et al.*, 1992).

The final significant pattern was the difference in fly abundance on sites that used different manure handling strategies, with fly catch being greater on the three sites with multiple temporary dung storage sites than the site with a single, permanent managed dung heap. This result must be treated with caution given that only one site had a centralised manure heap, and the statistical result may simply reflect other underlying management differences that affected the stable fly population, rather than any direct effect. The result also appears at odds with previous studies, which have found that stable fly larvae are only present in aged manure, greater than two weeks old (Broce & Haas, 1999; Cook *et al*., 2018).

Controlling stable flies is problematic due to their dispersal behaviour and the fact that they are not resident on the host. Maintaining and achieving effective coverage and long-lasting persistence of insecticides or on-animal repellents is difficult (Machtinger *et al*., 2016) and environmental treatment is undesirable on environmental grounds. Several studies have suggested that trapping might be an effective management strategy. However, while Alsynite traps are effective in capturing *S.calcitrans*, their upkeep may be costly and time-consuming. In order to prevent saturation with captured flies, frequent visits to replace the sticky sleeves are required, which may not be viable for many animal management facilities. Replacing the sticky sleeves is also costly, given that high densities of traps are needed to suppress a fly population.

After emergence, flies disperse from their larval development site before feeding, and movement beyond 5 km has been recorded (Taylor *et al.,* 2010). However on a local scale, stable flies are typically relatively immobile, moving only between hosts, resting sites and oviposition sites (Showler & Osbrink, 2015; Hafez & Gamal-Eddin, 1959). The current study suggests that where hosts are abundant, the relative attractiveness of traps may be lower than in areas where hosts are less abundant, because in the former case flies are strongly localised around their hosts. Hence, traps may not be a sustainable long-term solution for control at sites where high densities of stable flies are present.

In this study, traps were not accompanied by an odour bait. There have been numerous studies designed to improve Alsynite trapping, including supplementing Alsynite with treatments, and using alternative materials such as PET and coroplast (Beresford & Sutcliffe, 2006; Foil & Younger, 2006). Foil & Younger suggested that permethrin treated targets may increase the effectiveness of Alsynite trapping systems (2006), and Cilek found that adding a phenolic mixture (a 4:1:8 combination of 1 Octen-3-ol, 3-*n*-propylphenol, and 4-methylphenol) increased catches 6-fold; suggesting Alsynite trapping can be used alongside other methods (1999). Nevertheless, the addition of an odour-bait may be costly due to the volatility of the compounds used, leading to the need for frequent bait replacement, so the additional cost would need to be balanced carefully against the higher trapping efficiency achieved.

Th data presented here suggest that stable flies in south west England are highly seasonal and multiple factors affect their local abundance. Predicting the factors which affect fly abundance is problematic and a larger-scale study across multiple sites would be useful to achieve greater resolution of these factors. However, given the behaviour of stable flies in the presence of abundant hosts, the cost-effectiveness of trapping would require careful evaluation prior to general use.

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Figure legends

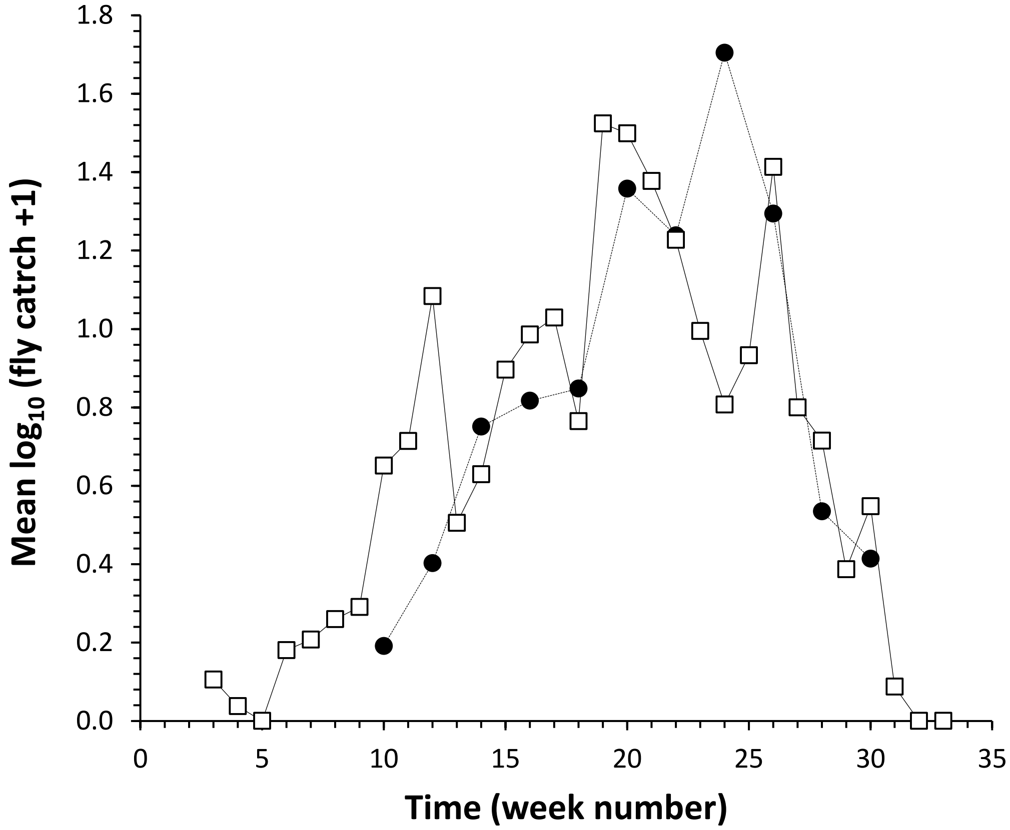
Fig. 1. The mean $\log_{10}$ number (+1) of *S. calcitrans* caught per week per trap over time (weeks) at four donkey sanctuary sites (1 to 4) in south west England in 2016 (solid circles, dashed line) and 2017 (open squares, solid line). Week 1 is the first week of May.

Fig. 2. The mean $\log_{10}$ number (+1) of *S. calcitrans* caught per week per trap at four donkey sanctuary sites (1 to 4) in south west England in 2016 (solid circles, dashed line) and 2017 (open squares, solid line).

Fig. 3. The RFU, scaled for index of body size, of male and female *Stomoxys calcitrans* of different ages reared in the laboratory. Females (F_{31}=1409, P<0.001, r^2=97.9, n=30, $Y=5.96.X^{0.744}$) (solid circles, dotted line) and Males (F_{24}=454.8, P<0.001, r^2=95.2, n=24, $Y=5.93.X^{0.823}$) (open circles, solid line).

Fig. 4. The frequency of *S. calcitrans* caught in 2017 of different chronological ages (2-day age classes) for males (open bars) and females (solid bars).

Fig 1



1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Mean log10 (fly catrch +1)
0
5
10
15
20
25
30
35
Time (week number)

Fig 2

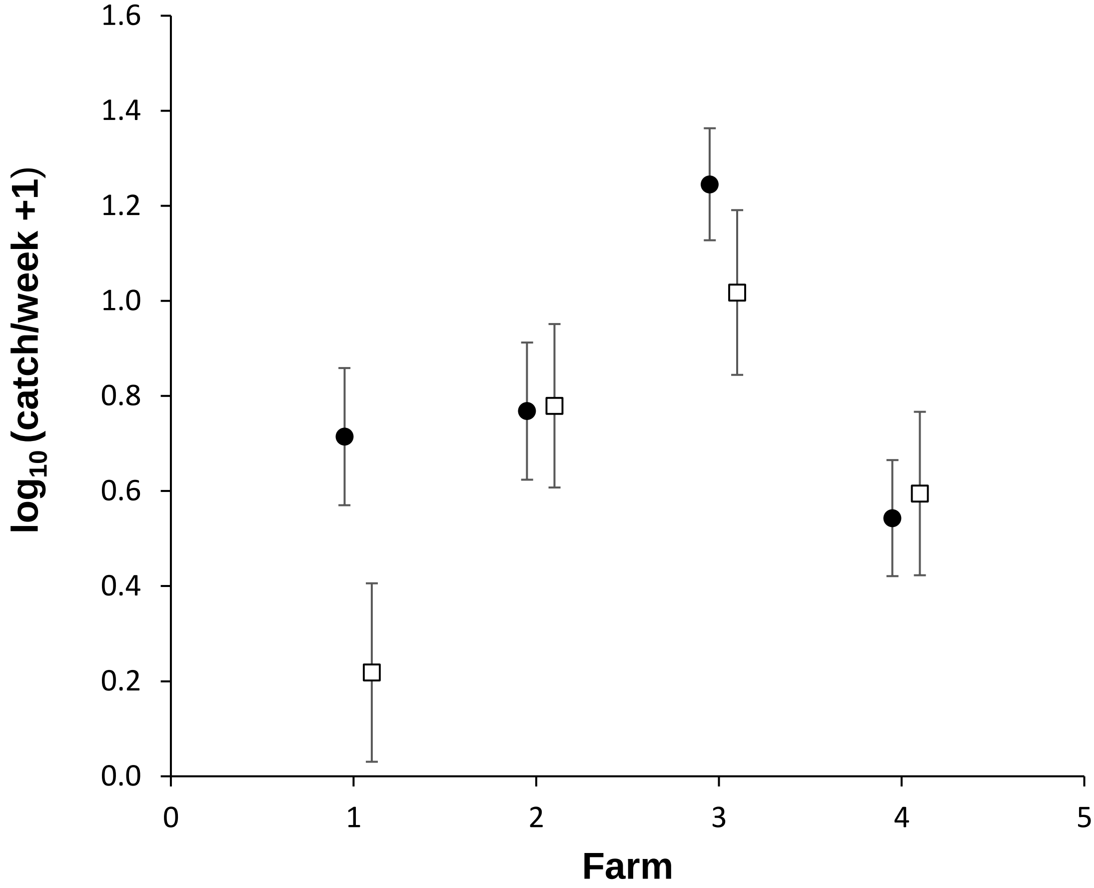
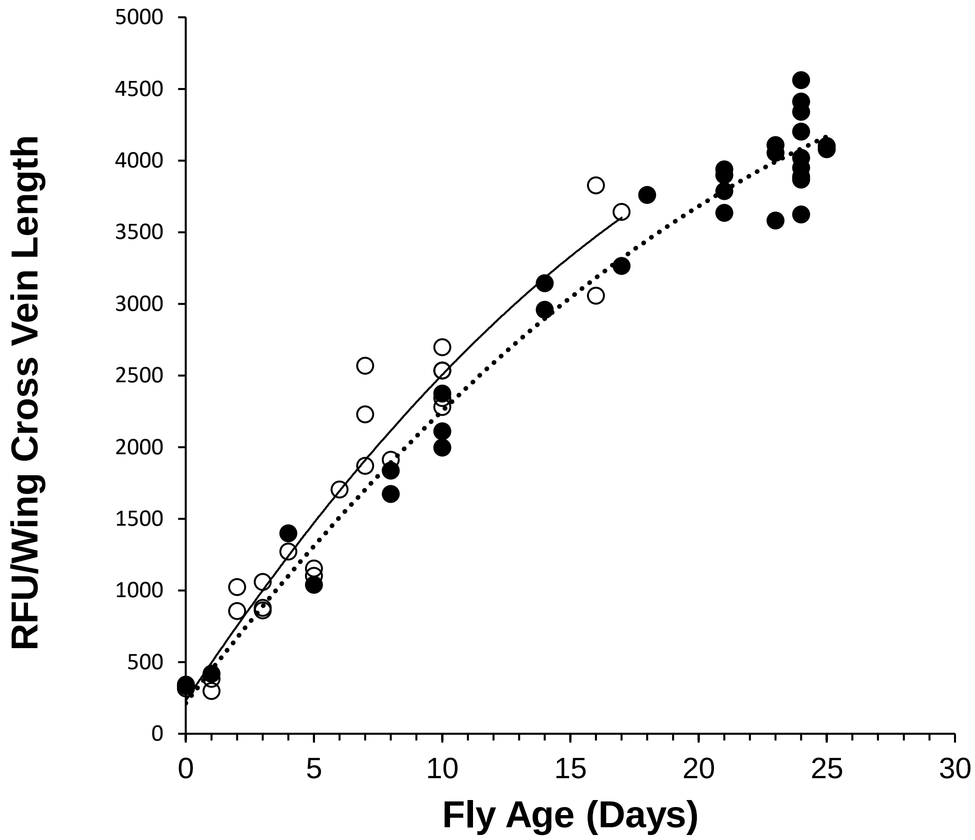
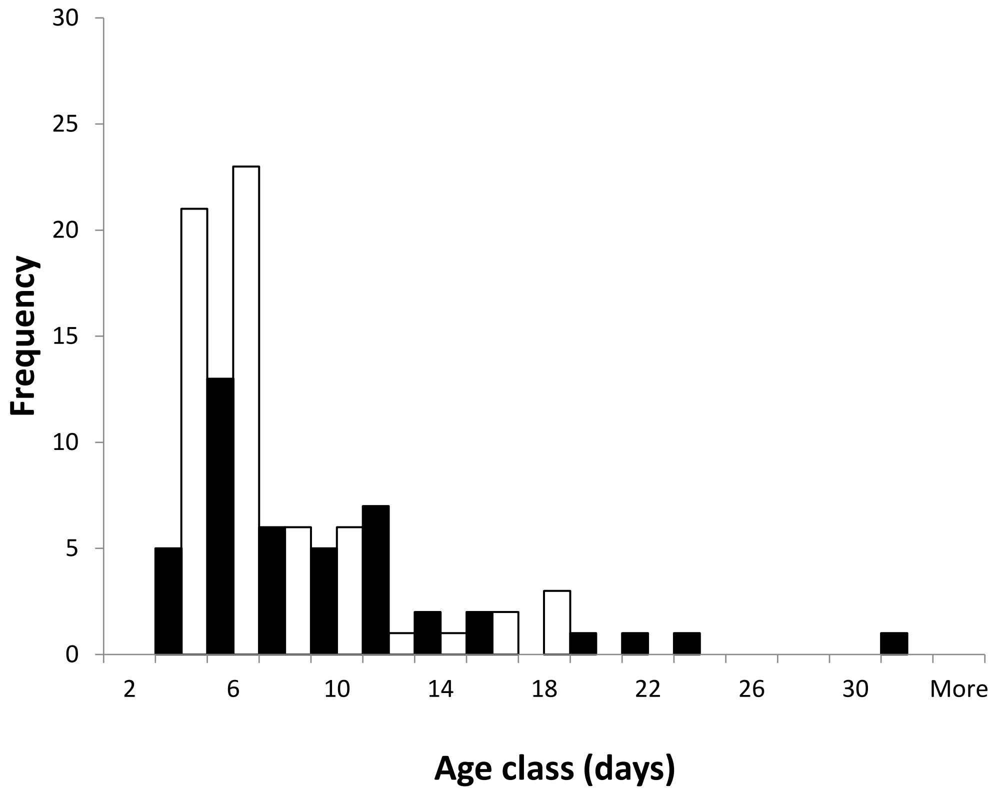


Fig 3



RFU/Wing Cross Vein Length
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
Fly Age (Days)

Fig 4


Frequency
30
25
20
15
10
5
0
2
6
10
14
18
22
26
30
More
Age class (days)